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A method for assessing the palatability of senesced leaf litter using *Folsomia candia* (Collembola: Isotomidae)

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With 3 figures

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1. Introduction

Many features of the chemical composition of senesced leaves determine the speed of their decomposition. The carbon: nitrogen ratio is frequently used as guide to decomposition speed (SWIFT *et al.*, 1979), although FEENY (1970) pointed out that a shortage of either carbohydrate or nitrogen would reduce the rate of decomposition. Whatever the C:N ratio, phenolic compounds, especially tannin, have been implicated in determining the palatability of leaves (SATCHELL & LOWE, 1967). Silica bodies, which are contained in most grasses (ESAU, 1953; METCALFE, 1960) are highly unpalatable (PARRY & SMITHSON, 1964) and it has generally been assumed that silica in leaves decreases their palatability.

Which features of leaf chemistry should be analysed if data on the differential rates of decomposition of a range of plant species are to be determined? Rather than undertaking series of chemical analyses, the aim of this study is to obtain an integration of all of the various chemical attributes by developing a laboratory test using a single insect species from the decomposer community. However, why might one wish to assay plant material for its palatability by decomposer animals? There are clearly 2 fields of research where it would be useful to obtain such information.

First, the information would be useful in screening a wide range of plant species (Anonymous,1985). It is not possible to test each individual plant species for its palatability by each of a range of herbivores, either because of the excessive resource requirements for such testing or due to the feeding specificities of many herbivores. Thus, in any kind of screening programme, the smallest possible number of generalist herbivores will be required, and their reactions to a species of plant will have to be taken as representative of many species of herbivore.

Second, such information is important in understanding the food webs in decomposer communities, communities that have hardly been studied (PIMM, 1982) from this point of view. COLEMAN (1985) underlined the need for a greater understanding of the decomposer part of terrestrial ecosystems.

In order to develop a screening procedure, a test animal needed to be selected. It should be a species susceptible to food quality and a generalist in its feeding habits. USHER (1985) stated that Collembola are useful in this respect as they are susceptible to food quality. MCMILLIAN (1975) analysed several soil-dwelling Collembola and found that all species contained plant, fungal and mineral material in their guts; this was taken to indicate generalist feeding habits. SHAW (1985) reported that *Onychiurus armatus* has fairly predictable responses to the defence chemistry of the fungi being eaten, and this might be expected for other species of Collembola as well. The ubiquitous (SALMON, 1964) collembolan *Folsomia candia* WILLEM was finally chosen because it is easily cultured in the laboratory (USHER & STONEMAN, 1977), it is parthenogenetic and it both breeds and grows speedily. Being a small herbivore, only very small quantities of leaf litter are required.

2. Materials and methods

Leaf material was collected from trees and from a wet hay meadow. Leaf litter of *Quercus robur* L., *Betula pendula* ROTH and *Sambucus nigra* L. was collected from trees growing on the campus of the University of York. Based on the work of SATCHELL & LOWE (1967), the expectation was that *S. nigra* would be more palatable than *B. pendula*, which would be more palatable than *Q. robur*. The wet hay meadow was located beside the River Derwent at Wheldrake Ings, North Yorkshire (grid references SE 701441). For species with large leaves were selected from this species rich community, namely *Sanguisorba officinalis* L., *Filipendula ulmaria* (L.) MAXIM, *Caltha palustris* L. and *Glyceria maxima* (HARTMAN) HOLMBERG. Only one prediction could be made about the species from the grassland community: *G. maxima* leaves would be the least palatable because these containing silica bodies (PARRY & SMITHSON, 1964).

The litter was gathered in the autumn of 1986 just before leaf fall (experiments were carried out during the subsequent winter). The woodland leaves were gently shaken from the trees. The grassland litter was gathered, after the leaves had discoloured, but before they had either fallen or lain on the ground. The leaves were air dried in the laboratory for a minimum of 2 weeks, ground to a fine powder in a micro-hammer mill, and stored sealed and dry until used.

Feeds were made by mixing the powdered leaf material with distilled water to make a paste. Powdered dry baker's yeast was added to the dry leaf material in a ratio 1:6 and 1:10 respectively in the different experiments,and thoroughly mixed before the water was added. Small pellets of this paste were placed on a sheet of polythene, frozen and stored at approximately $-20°C$ until used.

The collembolan used was *Folsomia candia* WILLEM *var. distincta* BANGALL. The culture was parthenogenetic and had been kept in laboratory conditions since 1968, during which time it had been fed on baker's yeast. In the experiments all of the animals had been standardized such that they had all hatched from eggs on the same day. The Collembola were cultured in plastic pill boxes, 5 cm in diameter, with a 5 mm deep base of plaster of Paris and charcoal (8:1) mixture (see USHER & STONEMAN, 1977). A smooth surface to the culture substrates was essential (see VAN AMELSVOORT, 1987, for details of achieving this surface) so that animals could be photographed. By keeping the substrate moistened with distilled water, the atmosphere within the pots was maintained at 90–100% relative humidity. The experiments were conducted at a constant temperature of 22°C. To prevent undue fungal or bacterial contamination of the substrate, uneaten food was removed each week and replaced with a new food pellet. Each culture contained 8 animals, this number being chosen as a result of preliminary experiments (VAN AMELSVOORT, 1987). Dead animals were removed and replaced by individuals from "sacrifice" cultures.

Two sets of measurements were made. First, the cultures were photographed and the lengths of the Collembola were calculated by projecting the negatives on to a screen, measuring the images, and comparing their lengths with standard lengths similarly photographed, projected and measured. When animals were very small, length was also measured by killing subsamples of the animals and measuring lengths under a microscope with a graticule. Second, development time from hatching from the egg to maturation was measured by checking to determine when oviposition commenced in culture pots. All experiments were replicated 5 times.

3. Results

3.1. Development of a technique

Subsequent to a series of preliminary experiments, an experiment was designed as a trial for an assaying technique. An even-aged group of animals was used; they were 11 d old and had all been fed together in one large vessel on baker's yeast. They had tripled their initial size (average length 0.91 mm, 95 % confidence interval ± 0.03 mm) and could be observed on photographs though they were not yet mature.

These were then used in a series of experiments with 4 leaf litters (*Quercus, Betula, Sambucus* and *Sanguisorba*), all mixed in a 6:1 ratio with yeast, as well as with 3 comparative feeding regimes (pure *Sanguisorba*, pure yeast and no food). Six replicates of each feeding treatment were originally set up, but one of these was used as a "sacrifice" replicate. The experiment was run from the 11th the 65th d of the animals' lives, and cultures were photographed so that lengths of animals could be measured.

The increases in length are shown in fig. 1. On the final day of the experiment the differences between the lengths of the animals feeding on the seven different food types were significant ($F_{[6.245]} = 360.4$); those with no food were shortest, followed with increasing length by those feeding on *Sanguisorba* (pure), *Quercus, Betula, Sambucus* and *Sanguisorba* (with yeast) not significantly different, and finally with yeast-fed animals being the longest.

All of the yeast-fed cultures had started to lay eggs by the 20th day, at which stage 12 of the 21 cultures fed with the leaf and yeast mixtures had also started to lay eggs. By the 24th day all of these 21

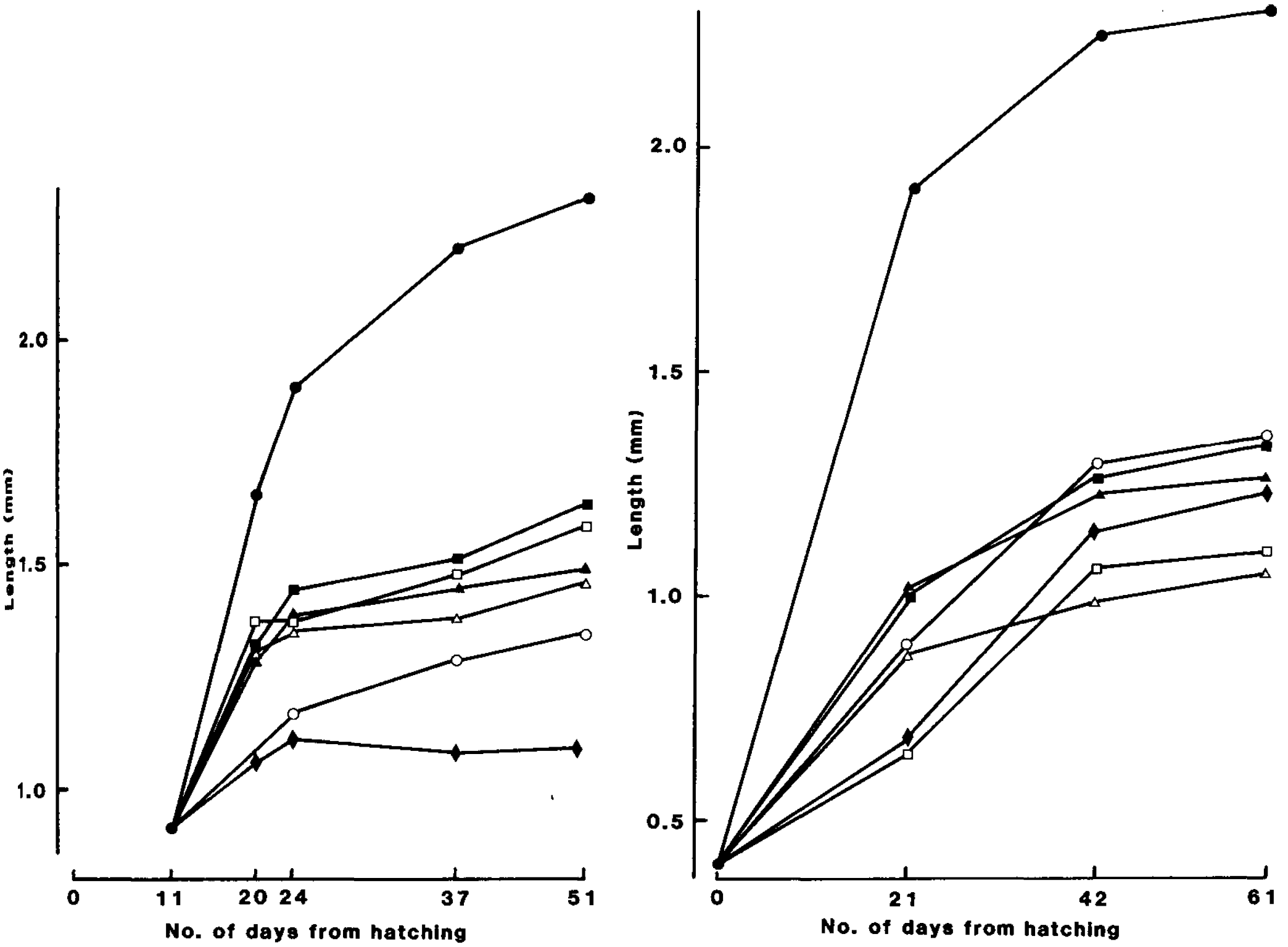

Fig. 1. The growth of *Folsomia candida* from the 11th to the 51st day after hatching from eggs. Cultures were fed on the following food types: ● yeast, ◆ unfed, ○ *Sanguisorba officinalis*, and in 6 : 1 mixture with yeast, ▲ *Betula pendula*, □ *Sambucus nigra*, △ *Quercus robur* and ■ *Sanguisorba officinalis*.

Fig. 2. The growth of *Folsomia candida* from the 1st to 61st day after hatching from eggs. ● represents cultures fed with yeast. All other cultures were fed with powdered leaf material in a 10 : 1 ratio with yeast. Leaf types are ○ *Caltha palustris*, ■ *Sanguisorba officinalis*, ▲ *Betula pendula*, ◆ *Filipendula ulmaria*, □ *Quercus robur* and △ *Glyceria maxima*.

cultures had eggs, and the first eggs were found in the pure *Sanguisorba* culture. All 5 cultures of the latter had eggs by the 37th day, whereas none of the cultures without food had eggs, even by the 65th day. Patterns of eggs production are mortality and discussed by van AMELSVOORT & USHER (1989).

3.2. A recommended technique

For the recommended technique 5 changes were made to the method described above.

(i) To gain greater differences in the time when oviposition first occurred, all experimental animals were fed on their experimental diet from their first day. Lengths of animals directly after hatching were measured using the microscope.
(ii) Based on the large differences between *Sanguisorba* pure and in a 6 : 1 mixture with yeast, the amount of yeast was reduced so that all leaf litters were mixed with yeast in a 10 : 1 ratio.
(iii) Culture pots were checked daily, from the 14th day, to record when oviposition first occurred. Daily observation stopped when oviposition had started.
(iv) The cultures were photographed every three weeks so as to determine the size of the animals.
(v) The experiments ended after 9 weeks, when oviposition had started in all replicates of each treatment, and when growth curves started to flatten.

Table 1. The mean lengths of *F. candida* (in mm) fed with 7 different types of food.

Food type	Mean length		Mean day to first oviposition
	after 6 weeks	after 9 weeks	
Glyceria maxima (H.) HOLMB.	1.00 a	1.06 w	41.0
Quercus robur L.	1.06 a	1.11 w	39.5
Filipendula ulmaria (L.)	1.15 b	1.23 x	34.5
Betula pendula ROTH	1.24 c	1.28 x	23.7
Sanguisorba officinalis L.	1.27 c	1.35 y	26.5
Caltha palustris L.	1.30 c	1.36 y	26.7
Yeast	2.26 d	2.31 z	18.0

Note: The leaf litter was in all cases mixed in a ratio of 10:1 with yeast. Within the columns, means with the same letter are not significantly different from each other.

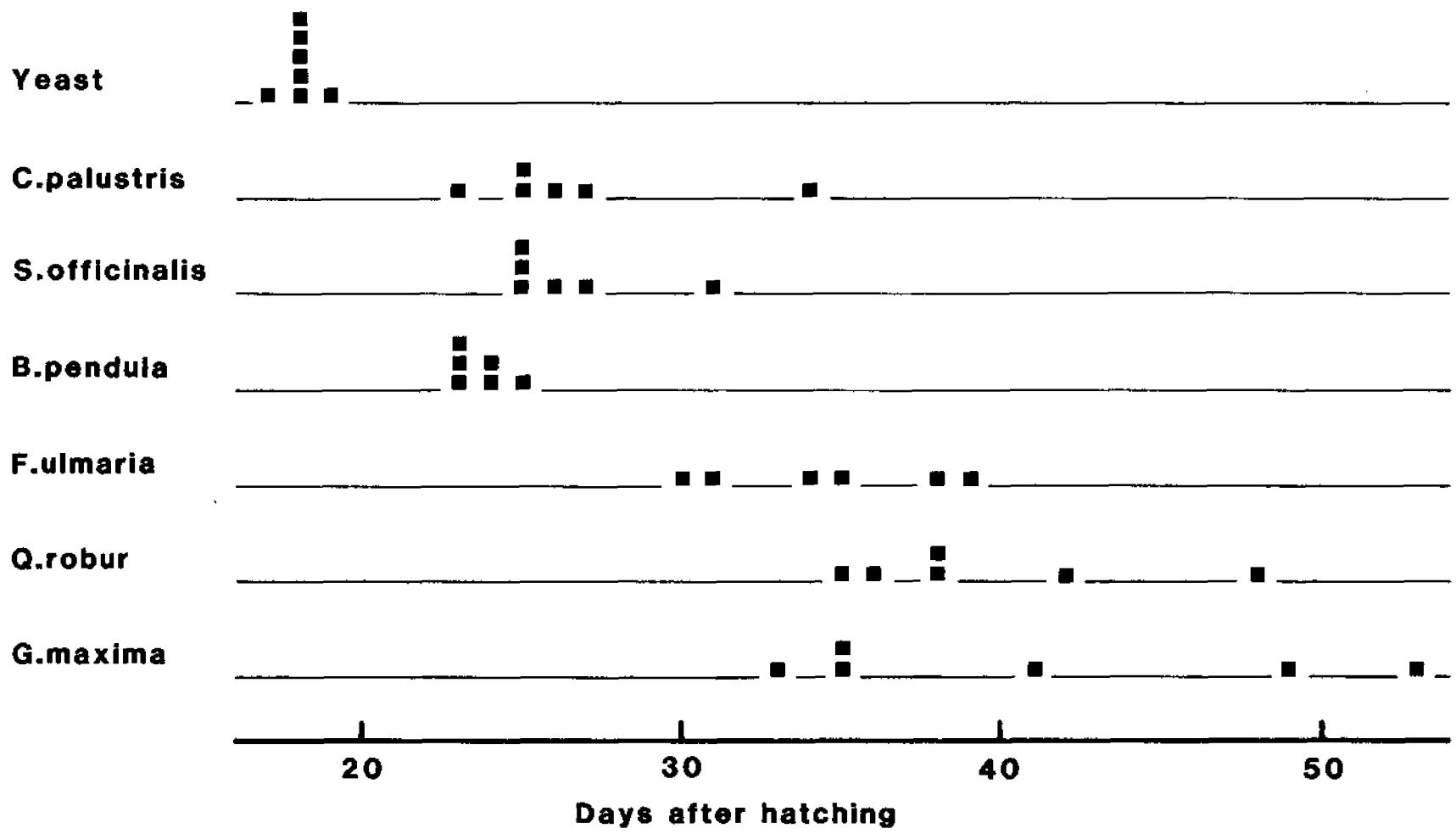

Fig. 3. The diagram shows the first day of oviposition in the cultures with the various food types portrayed in Fig. 2. Each symbol indicates an individual culture, and the horizontal scale gives the day after hatching from eggs when the first eggs were seen in the culture.

The growth of *F. candida* is shown in fig. 2. After 6 weeks there were significant differences between the lengths of the animals with the different food types ($F_{[6.245]} = 206.3$) and a significant interaction between food types and pots ($F_{[24.245]} = 3.45$). Similar results were obtained from the 9 weeks data ($F_{[6.245} = 200.6$ and $F_{[24.245} = 3.10$ respectively). However, as shown in table 1, there was a change in how the leaf litters differed from each other.

The time of first oviposition is shown in fig. 3 and the means are listed in table 1. The data have not been used in an analysis of variance due to the unequal variances. However, it is clear that the food types fall into 3 groups. First, the cultures fed with yeast were all ovipositing before any of the cultures fed with leaf material. Second, oviposition started after 23 to 25 d for cultures fed with *Betula, Caltha* or *Sanquisorba*. Third, first oviposition did not start till after 4 to 5 weeks for cultures fed with *Filipendula, Glyceria* or *Quercus*.

4. Discussion

In order to develop a useful technique a criterion, which demonstrates a clear reaction, to the investigated effect, is needed. BOOTH & ANDERSON (1979) used development (exuviae

production), fecundity (egg production) and mortality as such criteria in *F. candida*. KURUP & PRAHBOO (1982) used similar characteristics when investigating egg production in *Cryptopygus thermophilus*. In this study different test criteria were used because the relationship between food quality and both fecundity and mortality was complex (discussed in VAN AMELSVOORT & USHER, 1989) and a count of exuviae was felt to be inaccurate because exuviae are often eaten by the Collembola themselves and are difficult to observe. In comparison, both growth and onset of oviposition had a very clear and similar relation to food quality (palatability), as is shown in figs. 2 and 3, and they are therefore recommended as useful test criteria.

In the recommended technique leaf litters are offered to the animals in mixture with yeast. This was done for 2 reasons. First, because *F. candida* which had been fed on pure *Quercus* leaves never reached maturity (VAN AMELSVOORT, 1987). If the leaves were not mixed with yeast then differences between leaf types would be small and difficult to identify. Second, in field conditions leaves would be colonized by a range of microbes. In this assaying technique, this colonization was imitated by the addition of yeast, although there would also be other contaminants on the unsterilized litter that was used. The litter was not sterilized since sterilization, for example by heat, may alter the chemical nature of the leaves and it was expected that possible contaminants would have negligible effects. If not then these effects were expected to reinforce the results. TOUCHOT *et al.* (1983) stated that more palatable leaf litters support more microbes and that this influenced their feeding experiments with *F. candida* in the direction that was already expected. This influence has been fairly small, if not negligible, in the present experiments. Both HANLON & ANDERSON (1979) and INESON *et al.* (1982) reported the tendency that adding more *F. candida* to one culture inhibited microbial respiration. The population density of 8 animals per culture would, according to these authors inhibit microbial growth.

The results presented for the litter of tree species, especially the comparisons between *Betula* and *Quercus,* accord closely with expectations based on the reactions of the earthworm, *Lumbricus terrestris*, seen in SATCHELL & LOWE's (1967) experiments. Thus the indications are that the results may have relevance in a field situation. It is only after more extensive data have been collected, and comparisons with other generalistic, detritus-eating animals have been made, that a full appraisal can be carried out. However, the results of the wet hay meadow can be interpreted within the context of this technique. In table 1 it is shown that the growth rate of *F. candida* was minimal when it had to feed on *Glyceria*, significantly greater when feeding on *Filipendula*, and again significantly greater when feeding on either *Caltha* or *Sanguisorba*. Similarly, in fig. 3, the time of first oviposition was delayed longest when *F. candida* was offered *Glyceria*, delayed a week less when fed with *Filipendula*, and then more than a week sooner when fed with either *Caltha* or *Sanguisorba*. The apparently low palatability, as nutritional value, of *Glyceria* (comparable with *Quercus*) corresponds with the expectation based on the presence of silica in the leaves (PARRY & SMITHSON, 1964).

All of the indications are that *F. candida* responds to the array of defence chemicals, both volatile and involatile, found in the higher plants (HARBORNE, 1987). Different facets of its biology, either length growth of the animals or onset of oviposition (see table 1), are closely correlated so that essentially only one of these factors needs to be measured. Perhaps the greatest advantage of the technique described here is in the use of *F. candida*, which is a very versatile soil animal, a generalist feeder, and one that shows clear-cut responses to the litter of different higher plant species.

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Synopsis: *Original scientific paper*

Amelsvoort, P. A. M. van, & M. B. Usher, 1989. A method for assessing the palatability of senesced leaf litter using *Folsomia candida* (Collembola: Isotomidae). Pedobiologia **33**, 193–198.

A technique, based on length growth and first oviposition of *Folsomia candida*, was developed in order to measure the palatability of a wide range of leaf litters.

Animals were offered ground leaf litters, to which a small proportion of baker's yeast had been added, as food. Leaf litters from *Quercus robur, Betula pendula* and *Sambucus nigra* together with pure baker's yeast were used as reference feeds during the development of technique. The technique was applied to 4 species of a wet hay-meadow – *Sanguisorba officinalis, Filipendula ulmaria, Caltha palustris* and *Glyceria maxima*. Length growth was measured with the use of photographs, and rate of development was measured as the time from emergence from egg until onset of oviposition. Both showed significant responses to expected differences in the palatability of the leaf litters and were closely correlated.

The method could be used in screening plants for their palatability or for food web studies in the decomposer system.
Key words: Technique, Collembola, leaf litter, palatability, *Folsomia candida*, length growth, oviposition.

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